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Lactate accumulation rather than ATP depletion predicts ischemic myocardial necrosis
Implications for the development of lethal myocardial injury

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Abstract

In ischemia, the myocardial metabolic status determines the expansion of necrosis. Decreased ATP levels and increased lactate contents in ischemic myocardium undergoing lethal injury are known to be related to the expansion of irreversible damage. However, their individual contributions have not yet been firmly established. Using two differently effective protocols of ischemic preconditioning (IP short and IP long), ischemic cardioplegic arrest (CP) and their combination (IP+CP) to directly influence the metabolic status of porcine myocardium, graded preservations in ATP content and decreases in lactate accumulation during 45 min ischemia could be achieved (control: ATP, 0.15 ± 0.03; lactate, 60.53 ± 4.89 μmol/g wet weight; IP short, $0.33 \pm 0.10/27.42 \pm 3.90$; IP long, $0.60 \pm 0.10/17.49 \pm 2.14$; CP, $0.98 \pm 0.12/11.82 \pm 0.96$; IP+CP, $2.24 \pm 0.28/10.88 \pm 0.89$; all $P < 0.001$ vs. control). At the same time, a graded reduction of myocardial necrosis was observed (90.0 ± 3.1 vs. 31.7 ± 4.55 vs. 5.05 ± 2.1 vs. 0.0 [isolated patchy necroses] vs. none). Regression analysis revealed only a weak correlation of infarct size and ATP preservation ($r = 0.567$). In fact, there was a biphasic relation: with ATP levels above 1 μmol/g wet weight, no infarction occurred. ATP levels below this threshold value were associated with steep increase in infarct size. However, even for this latter range, the regression coefficient remained low ($r = 0.654$). Instead, over the entire range, there was a close, rectilinear correlation of infarct size and lactate accumulation ($r = 0.939$). These data indicate that lactate accumulation rather than ATP depletion determines the development of lethal myocardial injury. However, the biphasic relation between ATP depletion and infarct size suggests the latter to play a permissive role, since above a threshold value of 1 μmol/g wet weight neither substantial lactate accumulation nor infarction was observed. Below this threshold, however, infarct size increased as lactate accumulated.

Keywords: ATP; Lactate; Energy metabolism; Myocardial infarction; Myocardial ischemia; Infarct size

1. Introduction

The impact of the metabolic status of the ischemic myocardium on the extent of lethal myocardial injury is well established. On the basis of basic studies by Jennings and coworkers, the initiation of ischemic tissue injury is thought to primarily depend on two

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factors, i.e. ATP depletion and lactate accumulation [1]. Though both metabolic alterations are frequently observed in myocardium undergoing experimentally induced irreversible ischemic injury, controversy remains as to the individual contribution of each factor to tissue necrosis: in myocardium with decreased energy demand due to ischemic preconditioning (IP), no substantially altered time courses of ATP decline could be observed. Instead, a reduction in infarct size by about 50% could be more closely linked to a corresponding reduction in ischemic lactate accumulation [2]. In other studies, however, the removal of lactate from ischemic myocardium was not successful in limiting infarct size [3].

The re-evaluation of this long-standing controversy was the aim of our study. Choosing a stop-flow porcine model closely resembling human cardiac pathophysiology, IP, cardioplegic arrest (CP) and their combination were used to directly influence ischemic energy balance in order to delay the development of ischemic myocardial necrosis. By these established means, a graded reduction in ATP loss and in lactate accumulation as well as a graded reduction in irreversible myocardial injury were achieved. To the best of our knowledge, this is the first study to employ regression analysis on respective data in an effort to elucidate the individual contribution of each metabolic factor.

2. Materials and methods

The experimental protocol described in this study was approved by the Bioethical Committee of the District of Karlsruhe, Germany. Furthermore, all animals in this study were handled in accordance with the guiding principles for care and use of animals as approved by the American Physiological Society and the investigation conformed with the Guide for care and Use of Laboratory Animals published by the US National Institutes of Health.

2.1. Animal preparation

Thirty-one castrated German domestic pigs with body weights (BW) between 25.0 and 32.5 kg (27.8 ± 0.32 kg) were premedicated with 6 mg/kg BW intramuscular (i.m.) azaperone (Stresnil, Janssen Pharmaceutica, Neuss, Germany). Anesthesia was initiated 30 min later with 1 mg/kg BW midazolam (Dormicum, Roche, Germany) and 10 mg/kg BW ketamine (Ketanest, Parke-Davis, Germany) intravenously (i.v.). After endotracheal intubation, a bolus of 25 mg/kg BW of α-chloralose (Sigma, Deisenhofen, Germany) was given i.v. Anesthesia was maintained by a continuous i.v. infusion of 25 mg/kg BW/h α-chloralose. The animals were ventilated artificially with a pressure-controlled respirator (Pharmacia, Freiburg, Germany) with room air enriched with 2 l/min oxygen. Arterial blood gases were analyzed frequently to guide adjustment of the respirator settings. Additional doses of ketamine (10 mg/kg BW) were given i.m. every 60 min.

The left internal jugular vein was cannulated with polyethylene tubes for administration of saline, piritramid and α-chloralose. To measure aortic blood pressure, a 6F arterial sheath catheter was advanced into the aortic arch via the left common carotid artery and connected with a Statham transducer (P23XL, Statham, Puerto Rico). The chest was opened by a midsternal thoracotomy and the heart was suspended in a pericardial cradle. A loose silk ligature (3.0) was placed around the left anterior descending coronary artery (LAD) distal to the second diagonal branch and was subsequently tightened to occlude the vessel. After preparation, a stabilization period of 60 min was allowed before the different experimental protocols were started.

Index ischemia causing myocardial infarction was a 45-min LAD occlusion followed by 120 min of reperfusion. Ventricular fibrillation was immediately terminated by external defibrillation with paddles placed onto the external chest wall. Data from DC-amplifiers including surface-ECG were digitized by an analog–digital converter (Bisping, Germany) and continuously recorded onto the hard disk of a personal computer. Body temperature, as determined by rectal and intrathoracic measurements, was maintained within the physiological range by thermal isolation of the animals.

2.2. Experimental groups

The experimental protocol is summarized in Fig. 1. In all animals, sustained myocardial ischemia was

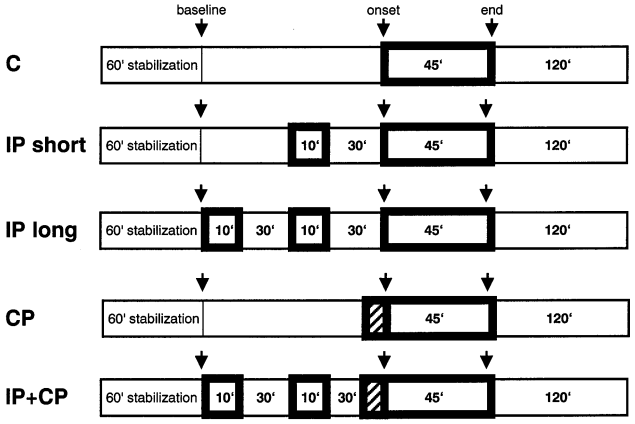

Fig. 1. Experimental protocol. LAD occlusions are indexed by solid-lined boxes, reperfusion periods by white bars. The numbers indicate the duration of these periods in minutes. Application of cardioplegia indexed by hatched boxes. Arrows mark the time points when myocardial biopsies were obtained (at baseline, before the onset and at the end of index ischemia). C, control; IP short, one cycle ischemic preconditioning; IP long, two cycles ischemic preconditioning; CP, cardioplegia; IP+CP, ischemic preconditioning (long) and cardioplegia.

achieved by a 45-min LAD occlusion (index ischemia). The animals of the control group (C, $n=6$) were only subjected to index ischemia followed by 120 min of reperfusion. In the second, so called IP short group ($n=6$), IP was achieved by one cycle of 10 min myocardial ischemia 30 min prior to index ischemia. In the third, IP long group, the animals were subjected to two ischemic episodes (10 min each, with 30 min of reperfusion in between) before the onset of the 45-min index ischemia (IP long). In the animals of the group subjected to cardioplegia (CP, $n=6$), CP was initiated according to standard methods [4] using 37°C Bretschneider HTK-Solution (Custodiol, Köhler-Chemie, Alsbach, Germany). Index ischemia was started once CP was achieved (15–20 s after onset of infusion). In the fifth group, the initiation of CP was preceded by a preconditioning protocol as performed in the IP long group (IP+CP, $n=6$). In the fourth and fifth groups, reperfusion was performed using retrograde aerobic perfusion at 80 mm Hg.

Myocardial drill biopsies were taken at the end of the stabilization period, i.e. before any experimental intervention (baseline), from virgin, non-ischemic myocardial tissue, directly before occluding the LAD at the onset of index ischemia, and at the end of this sustained ischemic episode. At the end of the experiments, myocardial infarct size was determined using the tetrazolium method [5], representing an established and valid method to visualize irreversible myocardial injury and to estimate infarct size [6,7].

Of the 31 animals instrumented, one animal was excluded because of ventricular fibrillation resistant to countershocking. All the remaining 30 animals successfully completed the experimental protocol.

2.3. Biopsies and metabolite analysis

Left ventricular drill biopsies representative of the ischemic area ($\approx$20 mg each) [5] were immediately frozen (within 2 s) and kept in liquid nitrogen until further use. Homogenization and deproteinization were performed in ice-cold 60% acetonitrile (v/v). Myocardial contents in ATP and lactate were assessed employing high-performance liquid chromatography [8,9] by an investigator blind to the results of the infarct size determinations (C.A.) and expressed as myocardial content in μmol/g wet weight.

2.4. Statistical analysis

All data are expressed as the mean and its standard error (S.E.M.). Statistical comparisons between groups were performed by analysis of variance (ANOVA). Different time points within one group were compared using repeated measurements ANOVA. A P value <0.05, as determined by Scheffé-testing, was considered statistically significant. Using bivariate plots, Lowess-curve fitting [10] (with tension set to 95%) was employed to qualitatively analyze dependencies between variables. For regression analysis, a linear model using ANOVA for the calculations of regression coefficients (r values) and statistical significances was chosen.

3. Results

3.1. Myocardial metabolite contents

For ATP content, the values at the onset of index ischemia did not – except for IP+CP – differ from

Table 1
Myocardial metabolite contents

Metabolite	Group	Baseline	Onset ischemia	End ischemia
ATP	C	3.95 ± 0.14	4.01 ± 0.17	$0.15 \pm 0.03^{\ddagger\P}$
	IP short	3.65 ± 0.22	3.32 ± 0.23	$0.33 \pm 0.10^{\ddagger\P}$
	IP long	3.61 ± 0.19	2.64 ± 0.29	$0.60 \pm 0.10^{\ddagger\#\P}$
	CP	4.24 ± 0.46	4.09 ± 0.36	$0.98 \pm 0.12^{\ddagger\P\#\S}$
	IP+CP	4.57 ± 0.55	$2.77 \pm 0.47^{\ddagger}$	$2.24 \pm 0.28^{\ddagger\#\S\beta\&}$
Lactate	C	2.93 ± 0.70	2.98 ± 0.59	$60.53 \pm 4.89^{\ddagger\P}$
	IP short	2.26 ± 0.76	3.14 ± 0.55	$27.42 \pm 3.90^{\ddagger\P\#}$
	IP long	2.69 ± 0.51	4.75 ± 1.42	$17.49 \pm 2.14^{\ddagger\P\#\S}$
	CP	4.26 ± 0.54	2.95 ± 0.25	$11.82 \pm 0.96^{\ddagger\P\#\S}$
	IP+CP	3.56 ± 0.51	3.35 ± 0.62	$10.88 \pm 0.89^{\ddagger\P\#\S}$

Myocardial metabolite contents for ATP and lactate. Significant differences as indicated: $^{\ddagger}P < 0.05$ vs. baseline; $^{\P}P < 0.05$ vs. onset ischemia; $^{\#}P < 0.05$ vs. C; $^{\S}P < 0.05$ vs. IP short; $^{\beta}P < 0.05$ vs. IP long; $^{\&}P < 0.05$ vs. CP. C, control; IP short and IP long, ischemic preconditioning employing the short and the long protocol, respectively; CP, cardioplegia; IP+CP, combination of ischemic preconditioning (long) and cardioplegia.

the baseline levels (Table 1, upper part). After index ischemia, ATP content was almost completely depleted in the control group, whereas this ATP loss was attenuated in the various intervention groups. Among these, a graded ATP preservation was seen: two cycles IP were more effective than one cycle, whereas CP did even better preserve ATP levels than IP. Best ATP preservation was observed in myocardium subjected to both, IP and CP.

As for ATP, myocardial lactate levels at the onset of index ischemia remained unaltered compared to baseline values (Table 1, lower part). During index ischemia, a marked increase in lactate content was observed, which was again attenuated in the intervention groups. For the various interventions, the order of graded attenuation roughly corresponded the pattern observed for ATP preservation.

3.2. Myocardial infarct size

With constant areas at risk for the five groups (Table 2), there was a graded reduction in myocardial infarct size with the two protocols of IP (IP long and IP short). In CP hearts, no contiguous necrotic area could be identified, though there were isolated, small patchy necroses in all animals. These could no longer be observed combining IP+CP. This graded reduction in irreversible myocardial injury seemed to run parallel with the degree of ATP preservation and inhibition of lactate accumulation (Table 1).

3.3. Regression analysis for metabolite contents and infarct size

Using bivariate plots to investigate the relationship between end-ischemic metabolite contents and ischemic injury (Fig. 2), the relation of ATP content and infarct size revealed a biphasic pattern: Above a value of about 1 μmol/g wet weight, myocardial infarction was not evident. Below this threshold value, however, a steep increase in infarct size was observed. The overall linear regression analysis (Table 3) between ATP content and infarct size revealed a regression coefficient of about 0.576. Even when limiting the analysis to data obtained below the threshold value, the r value only slightly increased (0.641).

In contrast to ATP preservation, the correlation between lactate accumulation and infarct size could

Table 2
Myocardial necrosis and infarct size

Group	RA/LV (%)	IA/RA (%)	Necrosis
C	17.2 ± 0.12	90.0 ± 3.1	transmural
IP short	16.2 ± 0.87	$31.7 \pm 4.55^{*}$	small
IP long	15.8 ± 0.72	$5.05 \pm 2.1^{*\P}$	discrete
CP	16.5 ± 1.11	$0.0 \pm 0.0^{*\S\P}$	patchy
IP+CP	16.9 ± 0.98	$0.0 \pm 0.0^{*\S\P}$	none

Area at risk (RA/LV), infarct size (IA/RA) and qualitative pattern of tissue necrosis. Significant differences as indicated: $^{*}P < 0.001$ vs. C; $^{\P}P < 0.05$ vs. IP short; $^{\S}P < 0.05$ vs. IP long; $^{\#}P < 0.05$ vs. IP+CP.

be well described using linear terms (Fig. 2). Linear regression analysis revealed a r value of 0.939 (Table 3).

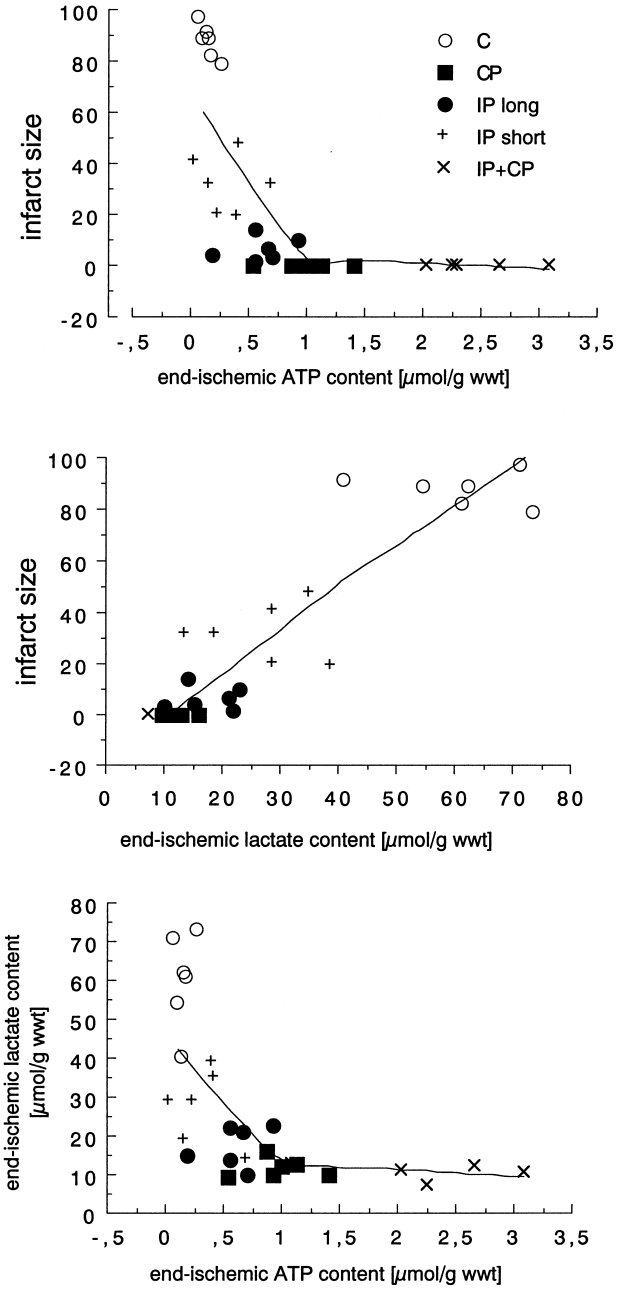

Fig. 2. Bivariate scatterplots demonstrating the dependency of infarct size from end-ischemic ATP content (upper graph) and from end-ischemic lactate content (middle graph), as well as the dependency of end-ischemic lactate content from end-ischemic ATP content (lower graph). Lowess-curve fitting (strength 95%) was employed to qualitatively analyze dependencies between variables.

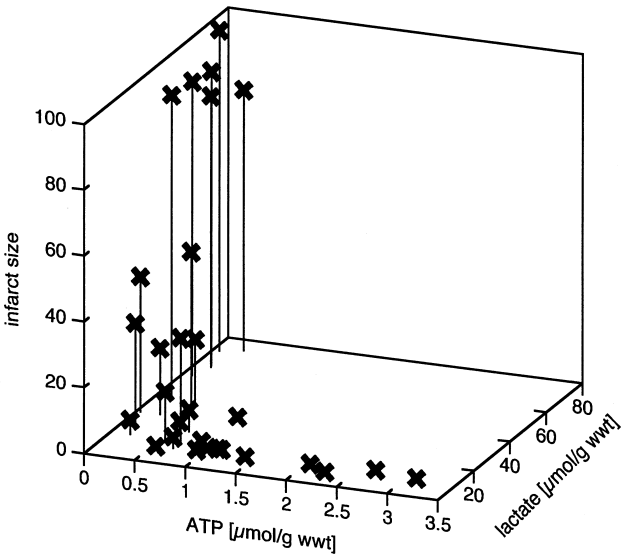

Fig. 3. Dependency of infarct size (vertical axis) from end-ischemic ATP content and end-ischemic lactate content (horizontal axes) in a 3D scattergram summarizing all experimental groups.

With a 3D scattergram summarizing the relationship of infarct size, end-ischemic ATP and lactate contents (Fig. 3), it becomes obvious that unless myocardial ATP contents fall below about 1 μmol/g wet weight, neither lactate accumulation nor relevant irreversible injury occurs. However, at ATP levels below this threshold value, marked and steep increases in end-ischemic lactate content and infarct size are evident.

4. Discussion

4.1. The expansion of tissue necrosis is accompanied by ATP depletion and lactate accumulation

The instantaneous breakdown of oxidative phosphorylation in acute myocardial ischemia results in a severe energy deficit [11]. Under this condition, even the immediate and marked stimulation of ATP formation by anaerobic glycolysis does not suffice to quantitatively cover myocardial energy demands [12]. In acutely ischemic myocardium subjected to reversible and then irreversible injury, decreased levels in ATP and increased myocardial lactate contents have frequently been observed and were held responsible for the expansion of myocardial tissue necrosis [1]. The data obtained in our control group confirm

Table 3
Regression analysis

Regression	Equation	r value	P
Infarct size (ATP)	$y = 46.346 - 24.425 \cdot X$	0.567	0.0006
Infarct size $(ATP)_{excluded}$	$y = 74.122 - 88.987 \cdot X$	0.641	0.0014
Infarct size (lactate)	$y = -15.21 + 1.584 \cdot X$	0.939	< 0.0001

Regression analysis for infarct size from end-ischemic ATP content including all data (upper row) and excluding data above end-ischemic ATP contents above 1 μmol/g wet weight (middle row) and for infarct size from end-ischemic lactate content (lower row). Regression equations, r values and P values (ANOVA) as indicated.

these basic observations, as markedly depleted ATP levels and largely increased lactate contents were found in myocardium undergoing transmural infarction.

4.2. Graded reduction in energy imbalance results in graded limitation of myocardial necrosis

Interventions which directly reduce myocardial energy deficit by limiting myocardial energy demands, such as IP [13], cardioplegia [12], hypothermia [14] etc., have repeatedly been shown to delay the expansion of lethal myocardial injury. Also in our study using 45-min index ischemia, two differently effective protocols of IP, ischemic CP, and their combination decreased ischemia-induced effects on ATP loss and lactate accumulation reflecting a diminished energy deficit. Accordingly, a graded reduction of ischemic tissue injury was observed.

4.3. Lactate accumulation, ATP depletion, or both?

Both, ATP depletion and lactate accumulation, are established indicators of severely ischemic myocardium developing tissue necrosis. As to the mechanism underlying irreversible myocardial injury, however, the individual contribution of each factor remains a matter of debate.

4.4. ATP depletion

Though a formally significant correlation between ATP preservation during ischemia and infarct size could be calculated, a r value of 0.567 indicates that the expansion of necrosis may only roughly be predicted by end-ischemic ATP content. Accordingly, the bivariate analysis did not show a linear dependence, but rather a biphasic relation of ATP content and infarct size. At first sight, these observations are in good agreement with Jennings' findings of an ATP level of about 0.4–1.0 μmol/g wet weight representing the critical threshold below which irreversible injury inevitably occurs [1]. However, even when only animals with end-ischemic ATP levels below this threshold were considered for regression analysis, the r value only slightly increased (0.641). Thus, even below threshold, ATP levels only weakly predicted the extent of irreversible injury. This finding is not easy to reconcile with the concept of the 'critical level of ATP' [1] in ischemia.

However, our findings are in line with published data providing evidence against the applicability of ATP preservation for direct prediction of tissue injury: using IP to reduce ischemic energy demands, Jennings and coworkers themselves could not convincingly explain the marked reduction in infarct size without a substantially altered ischemic ATP decline [2]. In their basic study, a more convincing relation could be observed between reduced ischemic lactate accumulation and infarct size reduction, both by about 50% [2]. Furthermore, other authors were able to show that myocardial ATP levels below the critical threshold do not inevitably result in tissue necrosis [15]. In fact, ATP levels can be reduced to extremely low values in myocardium not showing irreversible injury. Instead, these hearts may exert near normal contractile performance [16].

Thus, although depletion of myocardial ATP stores undoubtedly represents an important pathophysiologic factor for ischemic tissue necrosis, it remains doubtful whether these low ATP levels directly damage ischemic myocardium.

4.5. Lactate accumulation

The regression analysis between myocardial lactate accumulation and infarct size did reveal a highly significant correlation with a r value of 0.939. Different from ATP levels, a rectilinear response could be seen over the entire range. Since the r value found allows the prediction of the expansion of tissue necrosis with an accuracy of more than 90%, it can be assumed that lactate accumulation is directly involved in irreversible ischemic injury. This view is supported by published data indicating that an attenuation of ischemic lactate accumulation is beneficial for ischemic myocardium [17]. For example, in spite of a marked acceleration of ischemic ATP depletion, pharmacologic inhibition of anaerobic glycolysis was shown to attenuate lactate accumulation and not to result in premature manifestation of irreversible myocardial injury [18].

4.6. Summarizing considerations

To the best of our knowledge, this is the first study employing regression analysis in an effort to analyze the individual contributions of ATP depletion and lactate accumulation to irreversible ischemic injury. Our data indicate that ATP depletion by itself may not necessarily cause irreversible myocardial injury. In the development of myocardial necrosis, ischemic ATP loss may rather play a permissive role. The putative interaction of ATP loss, lactate accumulation and irreversible injury is summarized in Fig. 3: if myocardial ATP levels fall below 1 μmol/g wet weight, an increased ATP formation by anaerobic glycolysis occurs in order to compensate for the increasing energy deficit. With stop-flow ischemia, however, the beneficial effects of an increased ATP provision are rapidly counterbalanced by marked increases in the contents of potentially harmful metabolites and catabolites of the Embden–Meyerhof pathway. This results in a decreased tissue pH and an increased osmotic load, directly causing irreversible myocardial injury.

Hence, the limitation of lactate formation rather than the preservation of absolute ATP levels appears to be of benefit for severely ischemic myocardium. As our porcine model closely resembles human cardiac pathophysiology, these findings may be of importance searching for means to extend ischemic myocardial tolerance.

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